

LTNF PCR Amplification and Concentration Verification

Made with Benchling

Project: Awesome Possum

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Purpose: To amplify IDTDNA LTNF DNA constructs with decent concentrations for use in downstream applications.

Constructs:

- LT10
- LT10 + Promoter
- LT15
- LT15 + Promoter

Materials:

- Micropipette
- Sterile Micropipette tips
- PCR tubes
- 2x 1.5 mL eppendorf tubes
- 10.0 μ M LTNF Forward primer
- 10.0 μ M LTNF Reverse primer
- PCR Mastermix
- Construct DNA
- dH₂O
- Thermal Cycler
- EB Buffer
- Implen 300

Protocol:

A mixture of 240.0 μ L PCR Master-mix and 168.0 dH₂O was combined and gently spun to combine in a 1.5 mL eppendorf tube. Next, 24.0 μ L of LTNF Forward primer and 24.0 μ L of LTNF Reverse primer were combined and gently spun to combine in a 1.5 mL eppendorf tube. Then, 6 PCR tubes were labeled for each construct to be amplified (a total of 24 tubes). After that, 17.0 μ L of Master-mix/dH₂O mixture and 2.0 μ L of the primer mixture was pipetted into each of the 24 PCR tubes. Then, 1.0 μ L of each construct DNA was delivered to each correspondingly labeled PCR tube. Next, all PCR tubes were gently flicked to combine contents and were then placed into a thermal cycler.

The thermal cycler was set as following:

1. 95.0°C 3.0 minutes
2. 95.0°C 30.0 seconds
3. 62.0°C 30.0 seconds
4. 72.0°C 1.0 minutes
5. Repeat steps 2-4 for 12 cycles
6. 72.0°C 5.0 minutes
7. 4.0°C ∞

DNA Concentration Check with Implen P 300:

After PCR amplification was finished 2 tubes from each amplified construct group were analyzed for DNA concentration using the nanodrop function of an Implen P 300. The machine was blanked with 1.0 μ L of EB buffer before measuring concentrations. Then, a 3rd concentration for each group was obtained by combining all 6 PCR tube contents and then measuring. Please see table 1 for measurements obtained.

Table 1: DNA Concentrations of Amplified LTNF Constructs

	A	B	C	D	E	F	G
1	<u>Sample #</u>	<u>Concentration</u> <u>(ng/μL)</u>	<u>A230</u>	<u>A260</u>	<u>A280</u>	<u>A320</u>	<u>A260/A280</u>
2	LT10 Pro 1	440	0.081	0.176	0.101	0	1.743
3	LT10 Pro 2	438	0.079	0.175	0.1	0	1.75
4	LT10 Pro Combined	423	0.074	0.169	0.096	-0.001	1.76
5	LT10 1	442	0.08	0.177	0.1	0	1.77
6	LT10 2	445	0.079	0.178	0.101	-0.001	1.762
7	LT10 Combined	430	0.078	0.172	0.097	0	1.773
8	LT15 Pro 1	435	0.077	0.174	0.098	0	1.776
9	LT15 Pro 2	418	0.075	0.167	0.095	0	1.758
10	LT15 Pro Combined	410	0.075	0.164	0.093	0	1.763
11	LT15 1	448	0.081	0.179	0.102	0	1.775
12	LT15 2	453	0.081	0.181	0.103	0	1.757
13	LT15 Combined	430	0.081	0.172	0.099	0.001	1.737